Citation:

Taubert D, Roesen R, Lehmann C, Jung N, Schomig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: A randomized controlled trial. *JAMA* 2007; 298(1): 49–60.

PubMed ID: 17609490

Study Design:

Randomized controlled parallel-group trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To assess the clinical efficacy of low habitual amounts of cocoa for blood pressure (BP) reduction and to substantiate the hypothesis that cocoa phenol-stimulated nitric oxide synthesis is causative for BP lowering.

Inclusion Criteria:

- Both sexes
- Between 55 and 75 years of age
- In good general health except for upper-range pre-hypertension (BP between 130/85 and 139/89mmHg) or stage 1 hypertension (BP between 140/90 and 160/100mmHg)
- Not taking anti-hypertensive medications or nutritional supplements
- With normal plasma lipid and plasma glucose levels
- Non-manual workers or pensioners of higher socioeconomic status.

Exclusion Criteria:

Individuals were excluded if they had:

- Cardiovascular disease
- Diabetes mellitus
- Hyperlipidemia
- Gastrointestinal tract disease
- Hepatic and renal disorders
- Pulmonary disease
- Coagulopathy
- Cancer

- Psychiatric disorders
- Alcohol or drug dependence
- Seizure disorders
- History of organ transplantation
- Surgery within the last 12 months
- Positive test results for human immunodeficiency virus
- Hepatitis B or C
- A body mass index (BMI) of more than 27.5 or less than 18.5kg/m^2
- Actively smoked tobacco within the last five years
- Had regularly taken medications
- Had taken any medication within the last two weeks
- Used vitamin, mineral, or polyphenol supplements or food supplemented with biologically active compounds
- Were regular consumers of chocolate or other cocoa products of more than one serving per week.

Description of Study Protocol:

Recruitment

- Participants were unpaid volunteers recruited from a primary health care unit in Duisburg, Germany
- 119 volunteers responded to an invitation to participate in an 18-week study to "assess the relation of dietary habits and health status" and were screened by evaluation of medical history, physical examination, laboratory parameters and assessment of the individuals' habitual diet using a validated standardized semi-quantitative food-frequency questionnaire (FFQ).

Design

Eligible participants were assigned to the dark or white chocolate treatment groups by permuted randomization in sex stratified blocks of four persons each, sequentially allocated to dark chocolate and white chocolate using a computer-generated random number sequence.

Dietary Intake/Dietary Assessment Methodology

For accurate estimation of nutrient intake during the study, participants noted their actual daily food intake in precoded food diaries with 127 food items in 14 food groups adapted from the FFQ using common household measures to define standard serving sizes (e.g., slices, tablespoons, glasses).

Blinding Used

- To conceal group allocation from investigators, instructed trained staff at a separate site not involved with the trial generated and maintained the randomization list and prepared the chocolate
- Chocolate doses for each patient were wrapped in aluminum foil and provided in dated, sequentially numbered, sealed, non-transparent bags that transferred no information about the content
- All clinical investigations, dietary assessments, laboratory tests, data collection and data analysis were performed
  by physicians and trained staff who were blinded to group assignment

- Participants received no information about their examination data and the exact objective of the study until trial completion
- It was impossible to blind the participants to the intervention, because no polyphenol-free and polyphenol-rich chocolate of identical appearance was commercially available
- Also, removal of the polyphenols was obvious due to the loss of bitter and astringent taste
- Participants were instructed that disclosing their group assignment to investigators would result in exclusion from the study.

Intervention

After a cocoa-free run-in period of seven days and an overnight fast of 12 hours, participants were allocated to receive over 18 weeks either:

- 6.3g dose per day of commercially available polyphenol-rich dark chocolate containing 3.1g of cacao, (a total of 30mg of polyphenols, and 30kcal of energy) or
- Matching 5.6g dose per day of polyphenol-free white chocolate.

Statistical Analysis

- Normal distribution was assessed by the D'Agostino and Pearson omnibus normality test
- Pairwise within-group differences were assessed using the paired T-test and between-group differences by the unpaired T-test
- For greater statistical power between-group differences in outcome were also reported using an analysis of covariance adjustment of baseline imbalances
- For multiple pairwise comparisons, P-values were adjusted by the method of Holm
- Overall significance of differences comparing more than two measurements in the same individual was evaluated by repeated measures analysis of variance
- Linear correlation between two variables was assessed by the Pearson test
- The minimum sample size required to determine whether blood pressure was affected by the cocoa treatments was determined using the paired T-test by imputing a standard deviation of the change in systolic blood pressure (SBP) of 2.0mmHg and in diastolic blood pressure (DBP) of 1.5mmHg from previous intervention with 100g of dark chocolate over two weeks in a very similar population
- From studies with antihypertensive drugs, a decrease in SBP of 1.5mmHg and in DBP of 1.0mmHg was considered the limit for significant cardiovascular risk reduction. To detect this difference at a power of 0.8 with 95% confidence, a minimal sample size of 20 individuals in each group was calculated.
- All analyses were performed using SPSS version 11.0 and SigmaStat version 3.0 (SPSS Inc, Chicago, Illinois).

Data Collection Summary:

Timing of Measurements

- Adherence to the study protocol and the reported habitual food intake was confirmed in weekly visits by direct questioning, returning of the empty bags and assessment of food diaries
- Body weight; physical activity; plasma levels of lipids and glucose; and 24-hour urinary excretion of sodium, potassium, creatinine and nitrogen were assessed every six weeks
- Blood pressure and plasma parameters were assessed while each participant was in the 12-hour fasting state between 8 and 10 a.m. after the run-in period and after 6, 12 and 18

weeks of treatment
- To assess acute effects of dark and white chocolate, BP and plasma parameters were assessed in each participant at 0, 60, 120, 240, 360, and 480 minutes after the first chocolate dose following run-in and after another chocolate dose the day after completion of the 18-week treatment period

Dependent Variables

- Blood pressure: Measurements were performed in a noise-protected room of constant temperature (24°C) by trained, certified staff using a validated oscillometric device with appropriately sized cuffs (OmronHEM-722C, Omron, Mannheim, Germany)
- Bioactive Nitric Oxide: Venous blood was drawn into tubes containing EDTA and plasma was obtained by immediate centrifugation at 3000g for five minutes at 4°C, snap frozen in liquid nitrogen, and stored at −80°C until analysis. For measurement of S-nitrosoglutathione, following precipitation of proteins with acetonitrile, plasma samples were analyzed by liquid chromatography tandem mass spectrometry using positive-electrospray ionization by single-reaction monitoring of the precursor ion.

Control Variables

Not applicable.

Description of Actual Data Sample:

- *Initial N:* 119 Individuals screened for eligibility
- *Attrition (final N):* 75 excluded; Final N=44 (24 women, 20 men)
- *Age:* 56 to 73 years
- *Ethnicity:* White
- *Other relevant demographics:* Not applicable
- *Anthropometrics:* Not applicable
- *Location:* Germany.

Summary of Results:

Characteristics of Participants During Dark and White Chocolate Interventions [a]								
	Dark Chocolate Group, Mean (SD)				White Chocolate Group Mean (SD)			
	Change From Baseline by Week				Change From Baseline by Week			
Characteristics	6	12	18	P-value[b]	6	12	18	P-value[b]
Weight, kg	-0.08 (1.24)	0.09 (1.18)	0.13 (1.02)	0.84	0.12 (1.31)	-1.10 (1.15)	0.14 (1.13)	0.80
Body Mass Index	-0.02 (0.04)	0.03 (0.39)	0.04 (0.36)	0.87	0.04 (0.43)	-0.03 (0.38)	0.05 (0.38)	0.78

Blood Pressure, mmHg Systolic	-0.6 (1.6)c	-2.4 (1.4)d	-2.9 (1.6)d	<0.001	-0.1 (1.7)	0.4 (1.9)	0.1 (1.6)	0.71
Blood Pressure, mmHg Diastolic	-0.3 (1.1)e	-1.3 (0.6)d	-1.9 (1.0)d	<0.001	0.1 (1.9)	0.3 (1.7)	0.0 (1.8)	0.84
Heart Rate, beats per minute	0.2 (2.4)	0.3 (2.1)	0.1 (2.2)	0.71	0.1 (2.0)	0.1 (2.2)	-0.1 2.0	0.93
Physical Activity, exercises per day	0.0 (0.1)	0.0 (0.1)	0.0 (0.2)	0.95	-0.1 (0.2)	0.0 (0.2)	0.0 (0.2)	0.77
Cholesterol, mg/dL Total	-2.7 (2.3)	-2.7 (1.5)	1.2 (3.1)	0.82	2.6 (3.0)	0.8 (3.8)	-2.3 (3.1)	0.73
LDL	-2.3 (2.0)	-1.9 (2.2)	1.2 (2.3)	0.78	2.0 (4.2)	-1.1 (3.1)	-1.5 (2.8)	0.69
HDL	1.7 (1.5)	-0.8 (1.7)	1.3 (2.2)	0.85	0.4 (4.1)	-3.5 (2.3)	1.8 (1.5)	0.68
Triglycerides, mg/dL	-2.6 (6.2)	1.8 (9.6)	-4.4 (7.1)	0.71	3.5 (5.3)	-7.0 (6.3)	-1.8 (8.8)	0.79
Glucose, mg/dL	0.5 (1.1)	-0.6 (1.6)	-2.2 (2.5)	0.46	-0.3 (1.1)	-0.4 (2.0)	0.9 (1.5)	0.88
S-nitrosoglutathione, nmol/L	0.02 (0.09)f	0.19 (0.11)d	0.23 (0.12)d	<0.001	-0.01 (0.09)	0.00 (0.12)	0.01 (0.12)	0.85
Total 8-isoprostane, pmol/L	1 (10)	1 (11)	0 (9)	0.97	-2.0 (11)	-1 (10)	1 (12)	0.74

SI conversion factors:

To convert total, LDL, and HDL cholesterol from mg/dL to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113; glucose to mmol/L, multiply by 0.0555; creatinine to mmol, multiply by 8.84.

[a] All change values are normally distributed

[b] P-values of overall differences between treatment periods are calculated by one-way repeated measures of analysis of variance. $P<0.05$ indicates a significant difference between treatment periods. In the case of an overall significant difference ($P<0.05$), pairwise multiple comparisons are performed by paired T-tests and calculation of Holm-adjusted P-values with $P<0.05$ indicating a significant difference vs. baseline.

[c] $P=0.16$ vs. baseline

[d] $P<0.001$ vs. baseline

[e] $P=0.21$ vs. baseline

[f] $P=0.36$ vs. baseline

Author Conclusion:

- Small amounts of commercial cocoa confectionery convey a similar BP-lowering potential compared with comprehensive dietary modifications
- Whereas long-term adherence to complex behavioral changes is often low and requires continuous counseling, adoption of small amounts of flavanol-rich cocoa into the habitual diet is a dietary modification that is easy to adhere to and, therefore may be a promising behavioral approach to lower BP in individuals with above-optimal BP
- Future studies should evaluate the effects of dark chocolate in other populations and evaluate long-term outcomes.

Reviewer Comments:

A well-described study.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes

2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	Yes
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	Yes
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes

5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	Yes
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes

8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes